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Abstract: Williams-Beuren syndrome (WBS) is generally the consequence of an interstitial microdeletion at 7q11.23, which includes the elastin gene, thus causing hemizygosity at the elastin gene locus. The origin of the deletion has been reported by many authors to be maternal in 60% and paternal in 40% of cases. Segregation analysis of grandparental markers flanking the microdeletion region in WBS patients and their parents indicated that in the majority of cases a recombination between grandmaternal and grandpaternal chromosomes 7 at the site of the deletion had occurred during meiosis in the parent from whom the deleted chromosome stemmed. Thus, the majority of deletions were considered a consequence of unequal crossing-over between homologous chromosomes 7 (interchromosomal rearrangement) while in the remaining cases an intrachromosomal recombination (between the chromatids of one chromosome 7) may have occurred. These results suggest that the majority of interstitial deletions of the elastin gene region occur during meiosis, due to unbalanced recombination while a minority could occur before or during meiosis probably due to intrachromosomal rearrangements. The recurrence risk of the interchromosomal rearrangements for sibs of a proband with non-affected parents must be negligible, which fits well with the observation of sporadic occurrence of almost all cases of WBS

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ARTICLE

Unequal interchromosomal rearrangements may result in elastin gene deletions causing the Williams–Beuren syndrome

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Williams–Beuren syndrome (WBS) is generally the consequence of an interstitial microdeletion at 7q11.23, which includes the elastin gene, thus causing hemizygosity at the elastin gene locus. The origin of the deletion has been reported by many authors to be maternal in ~60% and paternal in 40% of cases. Segregation analysis of grandparental markers flanking the microdeletion region in WBS patients and their parents indicated that in the majority of cases a recombination between grandmaternal and grandpaternal chromosomes 7 at the site of the deletion had occurred during meiosis in the parent from whom the deleted chromosome stemmed. Thus, the majority of deletions were considered a consequence of unequal crossing-over between homologous chromosomes 7 (interchromosomal rearrangement) while in the remaining cases an intrachromosomal recombination (between the chromatids of one chromosome 7) may have occurred. These results suggest that the majority of interstitial deletions of the elastin gene region occur during meiosis, due to unbalanced recombination while a minority could occur before or during meiosis probably due to intrachromosomal rearrangements. The recurrence risk of the interchromosomal rearrangements for sibs of a proband with non-affected parents must be negligible, which fits well with the observation of sporadic occurrence of almost all cases of WBS.

INTRODUCTION

The Williams–Beuren syndrome (WBS) is characterised by growth and mental retardation with a friendly, outgoing personality, dysmorphic facial features, hypercalcaemia in early infancy, and congenital cardiovascular malformations, in particular supravalvular aortic stenosis (1–3). This, almost always sporadically occurring, disorder (incidence about 1/20 000 newborns) has been shown to result from a contiguous gene deletion syndrome comprising the elastin gene (4) and the recently located gene LIM-kinase 1 (5). Individuals with classic WBS have a chromosomal deletion >500 kb at 7q11.23 (5). Hemizygosity of the elastin gene is probably responsible for some of the characteristic features of the WBS, including supravalvular aortic stenosis, hoarse voice, and some of the WBS facial features. However, mental retardation and hypercalcaemia in WBS may indicate that hemizygosity of other genes adjacent to the elastin locus may also contribute to the phenotype.

The human elastin gene has been mapped to 7q11.23 (6). Indik *et al.* (7–8) found a remarkable abundance of *Alu* repetitive sequences in the 3′ region of the gene and several alternatively spliced isoforms.

Repetitive DNA sequences and *Alu* sequences flanking deletion breakpoints have been noted in a considerable number of human gene mutations which cause genetic disorders, including defects in the genes for α-globin (9), β-globin (10), growth hormone (11) and many others. Such repeated and highly homologous sequences may facilitate a misalignment of chromosomes followed by an unequal crossing-over event which increases the homologous recombination and greatly enhances the possibility of a deletion. Mispairing between the repeats might result in the formation of a loop between the two repeats. Subsequent excision of the loop will remove the sequences between the two repeats. One might hypothesize that an unequal recombination involving *Alu* sequences at 7q11 would generate a deletion resulting in WBS. The particular mechanism(s) involved in rearrangements of proximal 7q remains to be elucidated by molecular analysis of breakpoint sites.

In order to analyse the mechanisms underlying maternal and paternal *de novo* deletions in WBS, we performed a molecular study of 15 families. The parental origin of the deletion was first determined. Segregation analysis of 7q11–q21 markers in the 15 families with the available grandparents suggested that the deletions were in the majority due to an unequal interchromosomal rearrangement.

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Table 1. Results of molecular markers studies around the elastin locus in 15 WBS families

Family	Origin of deletion	Microsatellites			
		*ELN Mva*I	*ELN* dinuc.	*D7S489*	*D7S1870*
5	mat	b,ab,ab	b,bc,ab	**a,bb,aa**	**b,ac,bb**
9	mat	c,bc,ac	a,ab,ab	**a,bb,aa**	**b,aa,ab**
35	mat	a,ab,ab	**c,ab,cc**	**c,ab,bc**	
70	mat	**a,bc,ab**	b,ab,bb		
81	mat	a,bc,ac	b,ab,ab	a,bb,ac	c,ab,cd
82	mat	**c,ab,bc**	b,ab,bb	**a,bd,ac**	
84	mat	c,ac,bc	**a,bb,ab**	**a,bb,ac**	
101	mat	a,ac,aa	**b,ac,bc**	**b,aa,–**	**b,ac,ab**
3	pat	a,ab,cc	c,cc,ab	a,ab,bc	
34	pat	**b,ab,ac**	c,bc,ac	**c,cc,ab**	
38	pat	a,ab,ab	**a,ab,bb**	a,ab,ab	
41	pat	b,ab,bb	**a,ab,bb**	a,ab,ab	
52	pat	**a,aa,cc**	**c,ac,bb**	b,ab,bb	
63	pat	**c,ac,ab**	**a,ac,bc**	a,aa,aa	
76	pat	a,ab,aa	b,bb,ab	b,bc,ab	**b,bc,ad**

The alleles are given in the order: patient, mother, father. Markers informative for the parental origin of the deletion are shown in bold. Pat, paternal deletion; mat, maternal deletion. Families 3, 5, 9, 34, 35, 38, 41, 52, 63, 70 and 76 were also reported by Robinson *et al*. (30).

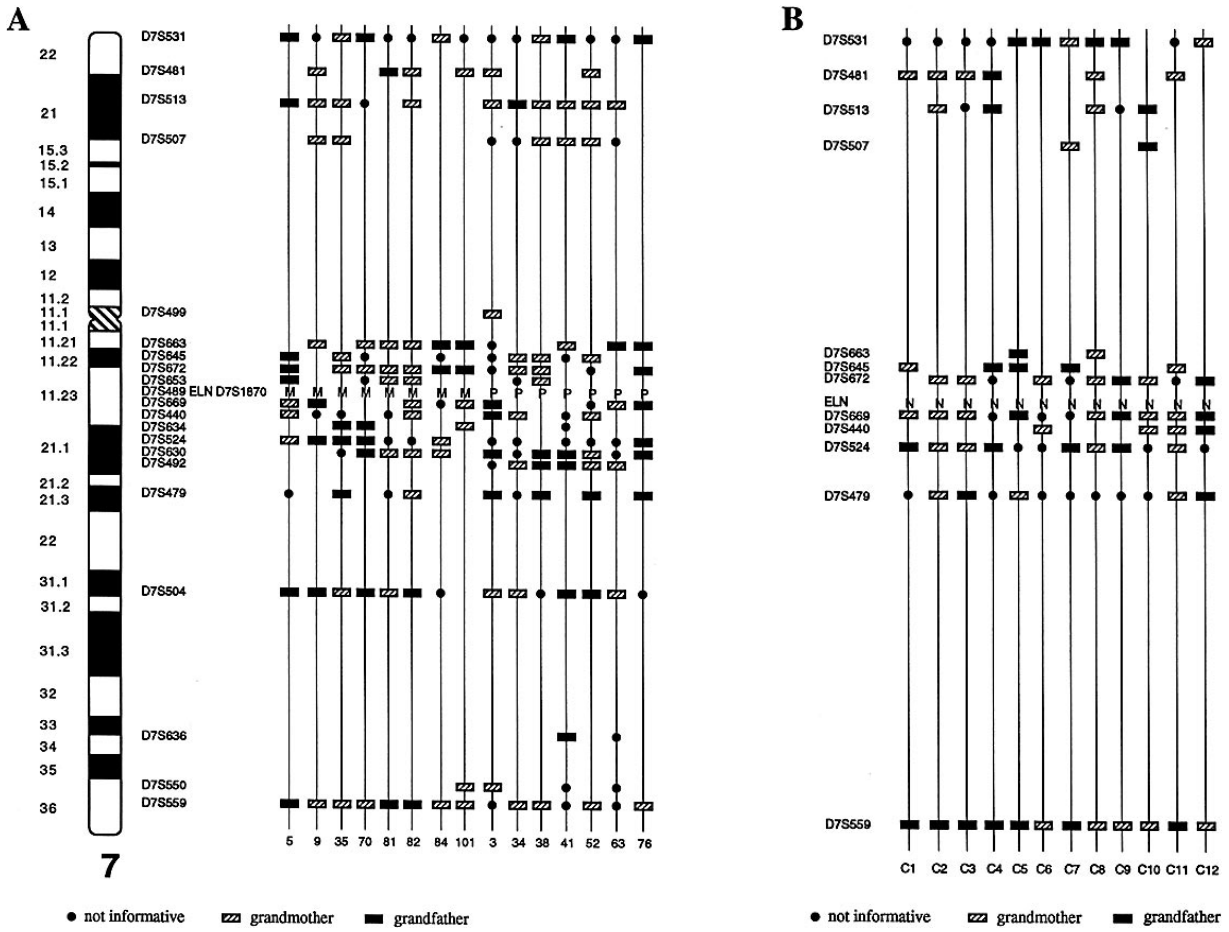

Figure 1. (**A**) Haplotype representation of chromosome 7 markers for WBS families with maternal (M) or paternal (P) deletion at the elastin gene. (**B**) Control families with normal (N) elastin gene. Alleles inherited from the grandmother are shown by hatched rectangles, markers inherited from the grandfather are shown by black rectangles. Closed circles represent uninformative markers.

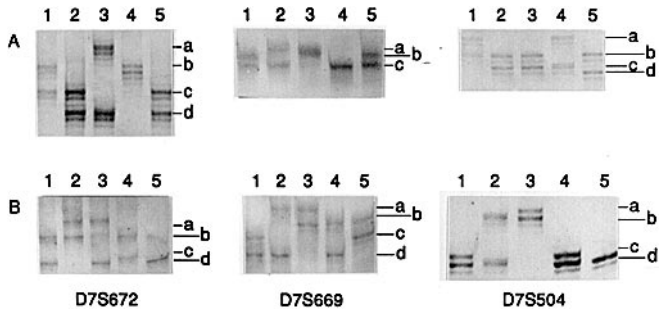

Figure 2. Results for the microsatellite loci *D7S672*, *D7S669* and *D7S504* in Family 5 (**A**) and Family 82 (**B**). The patient in Family 5 has inherited the grandpaternal allele c for *D7S672* and the grandmaternal allele c for *D7S669*, indicating crossing-over between markers flanking the deletion region. The patient of Family 82 has inherited the grandmaternal alleles b and d for the loci *D7S672* and *D7S669*, excluding crossing over, the grandpaternal allele d for *D7S504*. Lane 1, mother; lane 2, patient; lane 3, father; lane 4, maternal grandmother; lane 5, maternal grandfather.

RESULTS

The 15 WBS patients were genotyped for the elastin gene or for sequences flanking this gene. The absence of maternal or paternal contribution to the patients in at least one of these loci, demonstrated the origin of the deletion (Table 1). In eight cases there was no maternal inheritance of these markers, and in the other seven cases, there was lack of paternal alleles.

Fluorescent *in situ* hybridization (FISH) analysis of the parents, with the probe WSCR (ONCOR), demonstrated that all the deletions had occured *de novo*.

The 15 families consisting of the patient, the two parents and the available maternal or paternal grandparents were genotyped using microsatellites centromeric and telomeric adjacent to the elastin gene. The markers allowed us to determine which allele was inherited from the parents and from the grandparents. Haplotype analysis of the flanking markers on each side of the deletion showed a recombination event between the centromeric (*D7S663*, *D7S645*, *D7S672*, *D7S653*) and the telomeric loci (*D7S669*, *D7S440*, *D7S634*, *D7S524*, *D7S630*) in 10 out of the 15 analysed families (Fig. 1A). As shown in Figure 2A, in Family 5 the WBS patient inherited the allele 'c' from the maternal grandfather for locus *D7S672*. The allele 'c' for locus *D7S669* was inherited from the maternal grandmother, suggesting a crossing-over between the two reported loci, which caused the deletion at the elastin gene. In contrast, in Family 82 (Fig. 2B) the alleles 'b' (at *D7S672*) and 'd' (at *D7S669*) were inherited from the maternal grandmother. A recombination event between the grandmaternal and grandpaternal haplotypes in the deleted region is unlikely.

Buetow *et al.* (12) showed that the elastin gene maps between *D7S502* and *D7S524*. Biallelic inheritance was observed with the markers *D7S663*, *D7S645*, *D7S672*, *D7S669*, *D7S440*, *D7S634* and *D7S524* (Table 2), thus excluding hemizygosity at these loci for most of the WBS patients.

These results indicate that unequal crossing-over between the two chromosomes 7 is the mechanism causing the interstitial deletions in the majority of cases of WBS with elastin deletions.

No recombination of markers around 7q11.23 could be detected in the control families, who had no deletion of the elastin gene (Table 3 and Fig. 1B).

Since the 7q31–32 and 7p13 regions are frequently involved in rearrangements (12), we also investigated the recombination events occurring at the two telomeric ends. In seven WBS families we detected a second recombination event (Table 2, Families 5, 9, 35, 70, 101, 3 and 38) with the markers *D7S504*, *D7S550* and *D7S559* mapping between 7q31 and 7qter. One family (84) did not show recombination for *D7S559* and two families (41 and 63) were not informative. The results of analysis of the microsatellites *D7S507*, *D7S513*, *D7S481* and *D7S531* close to the 7pter region demonstrated an additional recombination in the short arm in Families 70, 84, 41 and 63. The five families (81, 82, 34, 52 and 76) with no evident crossing-over at the deleted region, showed recombination either at 7pter (Families 81 and 34) or 7qter (Families 81, 82, 52 and 76). Family 82 had a recombination starting at *D7S504* (Fig. 2B) for the 7q. Family 52 showed two recombinations, the first between *D7S479* and *D7S504*, the second at *D7S559* (Fig. 1A). The same markers were used for the control families; eight showed recombination either at 7qter or at 7pter (Families C2, C6, C7, C8, C9, C10, C11 and C12). Families C1, C3 and C5 had a crossing-over between *D7S524* and *D7S479*. The results for control families, indicate that the telomeric regions are more recombinogenic than the loci close to the elastin gene.

DISCUSSION

We have demonstrated that in 10 out of the 15 (67%) WBS families with a *de novo* deletion within 7q11.23, the segment flanking the deleted region contained recombined haplotypes. Therefore the deletion must be the result of an unequal crossing-over event between the chromosome 7 homologues during gametogenesis. An excess of meiotic recombination at 7q11.23 and at 7pter or 7qter, might partially explain the resulting deletion. The remaining five (33%) families showed no recombination at either side of the deletion. The genetic distance between *D7S653* and *D7S669* is 8 cM, and thus the probability of a double recombination is very low. It is possible that the deletions in the non-recombined families result from intrachromosomal rearrangements. Thus, elastin deletions causing the WBS might arise either by interchromosomal events (unequal crossing-over) or by intrachromosomal rearrangements. No sex specific occurrence of intra- or interchromosomal rearrangements was demonstrated (2/8 for maternal WBS, 3/7 for paternal WBS). This is in contrast with the preponderance of unequal crossing-over in males and the preponderance of intrachromosomal deletion occurring in females as proposed by Leguern *et al.* (14) for hereditary neuropathy with liability to pressure palsies (HNPP).

Meiosis is the basis for the production of haploid gametes from diploid cells, allowing sexual reproduction in eukaryotic organisms. Meiotic cells undergo DNA replication followed by the establishment of physical connections between homologous chromosomes. Paired chromosomes undergo genetic recombination followed by reductional division at meiosis I. The mechanism of meiotic recombination includes reciprocal breakage, exchange of DNA segments and rejoining of chromatids.

On the basis of observed recombination frequencies, meiotic recombination does not occur randomly along chromosomes, but seems to be more or less frequent in specific regions. The structure of chromatin is directly involved in recombination as demonstrated by lack of crossing-over in highly condensed heterochromatin (15). Unequal recombinations are mediated by related gene sequences or repetitive sequence elements, as

Table 2. Microsatellite analysis of chromosome 7 markers in 15 WBS families

	Maternal Deletions							
	5	9	35	70	81	82	84	101
	P,M,F,GM,GF	P,M,F,GM,GF	P,M,F,GM,GF	P,M,F,GM,GF	P,M,F,GM	P,M,F,GM,GF	P,M,F,GM,GF	P,M,F,GM,GF
D7S531	***b*c,*b*c,ac,bc,*b*b**	ab,ab,ab,bb,ab	ac,bc,aa,ac,bb	***a*a,*a*b,ab,bb,*a*a**	ab,ab,ab,bb	ab,bb,aa,bb,bb	ac,ab,cc,-,bc	ab,ab,aa,ab,ab
D7S481		ad,cd,ae,bd,cc			***a*c,*a*c,cc,bc**	cd,bd,bc,ad,bb		bd,ad,be,cd,ab
D7S513	***a*c,*a*b,bc,bb,*a*a**	ac,ac,ab,cd,ac	ad,ac,de,ac,bc	ab,bb,aa,ab,ab		ab,ab,ac,ab,ad		
D7S507		cd,de,bc,ad,ce	cd,bd,cd,dd,ab					
D7S663		bc,bd,ac,bb,ad		ad,bd,aa,cd,ab	ac,bc,ab,cc	bc,ab,ac,bc,ac	ac,bc,ad,bc,cc	c*e*,b*e*,ac,bd,d*e*
D7S645	***a*a,*a*b,aa,bb,*a*b**	aa,ab,aa,ac,ab	aa,ab,aa,aa,bb	ab,ab,aa,ab,ab			ab,ab,ab,aa,bb	
D7S672	c*d*,bc,ad,bb,c*d*		ab,bd,aa,bc,cd	de,ae,cd,de,ab	ab,be,ae,bd	ab,bd,ad,bc,dd	*a*c,*a*c,cd,bc,*a*e	*b*e,*b*c,de,ac,*b*d
D7S653	a*b*,*b*c,ad,ce,*b*f	bb,bc,bb,bc,ac	cc,ac,bc,cc,ac	ab,bb,ab,ab,bb	bc,ac,ab,cc	cd,bd,ac,ad,ab		
D7S669	ac,bc,aa,cc,bc	*a*c,*a*b,cc,bb,*a*c	bb,bb,ab,bc,bb	bb,ab,ab,ac,bb	bb,bb,bb,ab	ad,cd,ab,bd,bc	bc,bc,bc,ab,bc	ac,ab,bc,ab,bb
D7S440	be,ae,bc,de,aa	ac,cc,ac,ac,bc	ab,aa,ab,-,ab	cc,cc,cc,ac,bc	ab,aa,bb,aa	ac,ac,bc,ac,cc		
D7S634			*b*b,a*b*,bc,ac,*b*c	a*d*,*a*b,cd,bb,*a*c				ac,ab,ac,ab,bc
D7S524	ab,ab,aa,bb,ab	*a*b,*a*c,bb,cc,*a*b	b*b*,a*b*,bb,-,b*b*	b*b*,a*b*,bb,aa,b*b*	aa,aa,aa,aa	ab,bb,ab,bb,bb	ac,ab,cc,aa,bb	
D7S630			aa,aa,ab,aa,aa	c*c*,b*c*,bc,bc,a*c*	ab,ac,ab,aa	ab,bd,ae,bc,bd	ab,ac,bb,ab,ac	
D7S479	ac,aa,bc,aa,aa		c*d*,b*d*,cd,bb,a*d*		bc,ac,bd,ac	ab,bc,ad,bd,cc		
D7S504	*b*c,a*b*,bc,ac,*b*d	c*d*,b*d*,ac,bd,*d*d	bb,bd,be,be,de	*b*c,*b*c,ac,cc,*b*c	ad,cd,aa,bd	b*d*,c*d*,ab,cd,*d*d	ab,aa,ab,ab,ac	
D7S550								ab,bc,-,bb,ac
D7S559	*b*d,*b*c,cd,ac,a*b*	ac,ae,bc,ae,de	bc,bc,cc,bc,ac	ab,ab,aa,ab,aa	a*b*,*b*d,ad,cd	b*b*,a*b*,ab,aa,*b*b	ab,bc,ac,bb,bc	ac,ac,aa,bc,ac

	Paternal Deletions						
	3	34	38	41	52	63	76
	P,M,F,GM	P,M,F,GM	P,M,F,GM,GF	P,M,F,GM,GF	P,M,F,GM	P,M,F,GF	P,M,F,GM,GF
D7S531	aa,ab,aa,aa	aa,aa,aa,aa	ac,ac,bc,cc,bc	*a*c,bc,*a*c,cc,*a*b	ab,aa,bb,ab	ab,ab,bb,bb	*b*b,bb,*b*c,bc,a*b*
D7S481	bb,bb,ab,bc				ad,ab,bd,cd		
D7S513	cd,bc,ad,bd	*a*b,bc,*a*d,cd	ad,ad,cd,cd,bc	bc,ab,cd,bc,dd	aa,aa,ac,ab	ac,ac,cd,bd	
D7S507		bc,ac,bb,ab	ad,dd,ac,aa,bc	ad,bd,ac,ad,cd	bc,cd,bc,ab	bc,cd,bb,ab	
D7S499	ab,ad,bd,bc						
D7S663	cd,ac,bd,bd			ab,aa,bc,bd,ce		bc,bd,ac,bc	c*d*,cc,b*d*,ab,a*d*
D7S645	bd,ad,bc,bc	ab,bb,ab,aa	ab,aa,bc,ab,cd	ab,bb,aa,ac,ab	ad,ad,cd,bd		
D7S672	ab,ab,bb,bb	ab,bc,ad,ac	bc,bc,cd,bc,ad	cc,cc,bc,bc,ab	ab,ab,ac,ac	aa,ab,ab,ab	c*d*,cd,b*d*,bc,a*d*
D7S653	bb,bc,bb,ab	ab,aa,ab,ab	ac,ac,ab,ac,bc	bb,bb,ab,ab,ab	bb,bb,ab,ab	cc,ac,ac,bc	aa,ac,ab,ab,ac
D7S669	*a*c,bc,*a*b,bd	bb,ab,ab,bb	aa,aa,ab,ab,aa	bb,bb,bc,ac,bb	ac,ab,cc,cc	ad,ab,cd,bc	*a*b,bc,*a*b,bc,*a*c
D7S440	*b*c,ac,*b*c,cc	bc,ac,bc,ab	aa,ab,ab,bb,ab	ab,aa,bb,ab,bb	bb,bb,ab,bc		
D7S634			aa,ac,aa,ac,ab	bc,bc,cc,ac,bc			
D7S524	aa,ab,aa,ab	ab,aa,bb,bc	a*b*,ab,*b*c,cc,*b*b	ab,ab,bb,bb,bb	aa,aa,aa,aa	ab,ac,bb,bb	*a*b,bb,*a*b,bb,*a*a
D7S630	b*b*,bc,a*b*,ac	aa,ab,aa,aa	a*c*,ab,bc,ab,*c*c	b*c*,bb,a*c*,aa,*c*c	aa,ac,ac,ab	bb,bb,ab,ab	a*c*,aa,b*c*,bb,a*c*
D7S492	bc,ac,bc,bc	c*c*,bc,a*c*,bc	*b*c,bc,a*b*,ad,*b*c	bc,bc,a*c*,aa,*c*c	ac,ab,bc,cc	bc,bb,ac,ab	
D7S479	b*d*,bd,a*d*,ac	bb,ab,ab,ab	bc,bb,b*c*,bc,a*c*		a*a*,ac,*a*b,bc		a*c*,aa,a*c*,ad,b*c*
D7S504	cc,cc,bc,ac	ab,bc,a*d*,ab	ab,bc,aa,ac,aa	*b*d,cd,*b*c,ac,*b*d	c*c*,cc,a*c*,ab	bb,ab,ab,aa	bc,bc,bc,bc,ac
D7S636				c*d*,ce,a*d*,ab,a*d*		ab,ab,ab,ab	
D7S550	ad,cd,ac,ab			aa,aa,aa,ab,aa		bb,bc,ab,ab	
D7S559	ac,cd,ab,ab	cd,dd,ac,bc	cc,bc,ac,cd,ab	ab,ac,bb,ab,ab	ab,ac,ab,bc	bc,ab,bc,bc	aa,aa,ab,aa,bb

The order of the family members available for genotype analysis is always indicated under the family number. Allele designations (marked a–f) are arbitrary. P, patient; M, mother; F, father; GM, grandmother; GF, grandfather. Bold indicates allele inherited from the grandmother. Bold italic indicates allele inherited from the grandfather. Underline indicates biallelic inheritance for markers flanking the elastin gene.

proposed by the presence of repetitive DNA sequences and *Alu* sequences flanking deletion breakpoints in many human genetic disorders (9–11). Misaligned non-sister chromatids during meiosis and misaligned sister chromatids, followed by excision of a chromatid loop, have also been proposed as a mechanism for deletions of several Mb such as those in the Prader–Willi/Angelman syndrome (16) and HNPP (14). The most abundant repetitive elements in the human genome are the *Alu* repeats, they have also been shown to be involved in a considerable number of human gene deletions (17–25).

The human elastin gene has relatively large introns which are characterized by *Alu* repetitive elements clustered at the 3′ end (7). The presence of repetitive elements might facilitate chromosome 7 misalignment in the elastin gene region. Furthermore, the breakpoints in the LIM-kinase1 gene, which is adjacent to elastin and directly involved in the partial WBS phenotype, seem to occur within *Alu* repeats (5). This finding strengthens the argument that *Alu* sequences are involved in unequal recombination leading to WBS deletion. Additional work is required to characterize the precise sequence at the deletion breakpoints of

Table 3. Microsatellite analysis of chromosome 7 markers in 12 control families

	C1	C2	C3	C4	C5	C6
D7S531	ab,ab,aa,ab,ab	ab,bb,ab,ab,ab	ac,bc,aa,ab,ac	ac,aa,bc,bc,bc	*a*a,aa,*a*b,bb,*a*a	*a*c,*a*c,bc,bc,*a*c
D7S481	bd,cd,ab,bd,aa	bc,bd,cd,ac,cd	cd,bc,cd,de,ac	bc,bb,ac,ab,cc		
D7S513		cd,cd,ac,bc,aa	bc,bd,cc,ce,ac	*b*c,ac,*b*d,cd,a*b*		
D7S663					cc,bc,a*c*,aa,a*c*	
D7S645	bd,ad,bc,bc,ac			a*b*,bc,a*d*,bd,a*b*	*c*e,de,b*c*,bb,a*c*	
D7S672		bd,bc,ad,dd,ac	bc,ab,bc,cc,bc	ac,ac,ac,ad,bc		bc,ac,bd,cf,ae
D7S669	ab,ac,bc,ab,cc	ce,be,cd,ac,cd	ab,ac,bc,bb,ac	ab,ab,ab,ab,ac	a*b*,bd,a*c*,cc,a*c*	ab,bb,ab,ab,ab
D7S440						cd,bc,dd,cd,ab
D7S524	a*a*,ab,a*b*,bb,a*a*	ab,bb,ab,aa,bb	bc,cc,ab,bc,ab	a*b*,bb,a*b*,bc,a*a*	ab,ab,ab,ab,ab	bb,bb,bb,ab,bb
D7S479	ab,aa,bb,bb,bb	de,bd,ce,ce,ac	a*c*,bc,a*b*,bb,a*c*	ab,ab,ab,bb,aa	cd,bd,ac,ac,ab	ab,ab,ab,bc,ab
D7S559	b*b*,ab,a*b*,aa,b*b*	b*c*,ab,b*c*,b*c*,ac	a*d*,cd,a*c*,bc,a*d*	a*c*,cc,a*d*,ad,a*b*	d*d*,ad,c*d*,ac,b*d*	bb,bc,bb,bb,ac
	C7	C8	C9	C10	C11	C12
D7S531	bb,bc,bb,bc,ac	a*d*,a*c*,ad,ac,a*b*	*b*b,a*b*,bb,aa,a*b*		dd,dd,ad,cd,bd	ac,bc,ac,-,ab
D7S481		bc,ac,bc,bc,ab			cc,cd,cc,ac,bd	
D7S513		ab,bc,ab,ab,bc	bb,bb,bb,ab,-	a*b*,a*b*,aa,aa,a*b*		
D7S507	bd,ad,bb,dd,ac			*b*c,a*b*,cc,ac,*b*b		
D7S663		ce,de,ac,ee,bd				
D7S645	a*c*,a*b*,ac,bb,a*b*				bc,bc,cc,bd,ac	
D7S672	cd,cd,cd,ad,bc	bb,bc,bd,ab,ce	e*f*,b*f*,ae,bc,d*f*	bb,ab,bb,bb,ab	bb,bb,bc,bb,ab	*c*e,cd,ef,ad,b*c*
D7S669	ab,aa,ab,aa,ab	bc,bc,bb,ac,bb	*b*d,*b*c,ad,bc,a*b*	bc,ac,ab,bc,ad	ab,bc,ad,bc,cd	a*c*,a*b*,bc,bc,a*b*
D7S440				ab,bc,aa,bb,bc	ac,bc,ac,bc,bd	a*b*,a*b*,aa,ab,*b*b
D7S524	a*b*,*b*d,ac,dd,*b*b	ac,ab,bc,ac,bc	*b*b,a*b*,bb,ab,*b*b	aa,aa,aa,aa,aa	ab,ab,bb,aa,bb	bb,bb,ab,bb,ab
D7S479	ac,cc,ab,cc,cc	aa,ab,ac,ab,ab	bb,bb,bc,bd,ab	bb,bb,ab,bc,bb	bc,ac,bd,bc,ae	a*b*,a*b*,bb,bb,a*a*
D7S559	a*c*,a*c*,bc,bc,a*b*	bb,bc,ab,ab,bc	ac,ab,ac,aa,bb	ce,cd,ce,ac,bd	b*d*,a*d*,bd,ac,*d*d	ad,ac,bd,ad,ac

The order of the family members for genotype analysis is always child, mother, father, grandmother, grandfather. Families C1–C5, paternal grandparents; C6–C12, maternal grandparents. Allele designations (marked a–f) are arbitrary. Bold indicates allele inherited from the grandmother. Bold italic indicates allele inherited from the grandfather.

classical WBS (>500 kb), in order to determine the pathogenetic mechanism(s) that cause these (and other) deletions.

Unequal crossing-over during meiosis was also suggested as a mechanism generating DNA duplication on chromosome 17 causing Charcot–Marie–Tooth disease, and the deletion in the same region causing HNPP (26). Duplications and deletions have been also described in the LDL receptor gene (17). For the elastin gene or the 7q11.2 region there is no description of patients with a duplication. In addition to the difficulty in detecting such a duplication with an unknown phenotype, it is possible that the corresponding duplication is either not associated with an abnormal phenotype or that it is lethal.

In the control families, recombinations had mainly occurred at the two chromosome 7 telomeres and not at the elastin locus. Genotyping of these few control families seems to suggest that the chromosome segment 7q11 is not especially prone to recombination. It may be expected that a lower recombination frequency occurs in regions where an intact structure of both alleles must be maintained for correct gene expression.

A practical consequence of our findings is that they can improve prediction of recurrence risk for sibs of an affected proband. If a recombination around the deleted segment can be demonstrated, the deletion must have occurred during meiosis; mitotic recombination is extremely unlikely, and thus the recurrence risk will be virtually zero. In case of a presumed intrachromosomal recombination, an unlikely premeiotic origin cannot be fully excluded. Thus, the possibility of gonadal mosaicism for the deletion has to be considered, with the recurrence risk possibly being in the range of 1–2%. Occurrence of WBS in sibs of unaffected parents has been reported earlier (27), but to the best of our knowledge has never been reported in cases with proven elastin deletion. On the other hand, there is one report of a 22q11.22 microdeletion in sibs of non-deleted parents (28). It would be interesting to investigate this family for recombinations around the deletion.

Prader–Willi/Angelman syndrome and CATCH22, like WBS, are genetic diseases characterized by interstitial deletions close to the centromere. Segregation analysis using markers flanking the microdeletion region in these three conditions might provide further information as to whether a common mechanism is responsible for interstitial deletions.

MATERIALS AND METHODS

The patients were selected from a series of WBS patients investigated with microsatellite markers and FISH at the Institute of Medical Genetics of the University of Zürich; clinical and molecular data have been reported by Kotzot *et al.* (29) and Robinson *et al.* (30).

DNA was extracted from peripheral blood of the probands with WBS, from each parent, and from the grandparents from the side of the deletion origin, using standard methods.

The following microsatellites with the indication of genetic order and distance (cM) were used: 7pter–*D7S531*-(11)-*D7S481*-(11)-*D7S513*-(8)-*D7S507*-(51)-*D7S499*-(3)-*D7S663*-(3)-*D7S645*-(3)-*D7S672*-(1)-*D7S653*-(1.8)-*ELN*-(6)-*D7S669*-(2.4)-*D7S440*-(0.6)-*D7*

S634-(6)-*D7S524*-(2)-*D7S630*-(3)-*D7S492*-(9)-*D7S479*-(26)-*D7S504*-(51)-*D7S550*-(4)-*D7S559*–7qter (31).

The following markers were used in order to determine the origin of the deletion: (i) *ELN Mva*I, the amplified products of exon 20 of the elastin gene were digested with *Mva*I as described (32); (ii) *ELN* dinuc., which maps near exon 18 within the elastin gene (33); (iii) *D7S1870* and *D7S489* with an informativity of 85% for detecting deletions at 7q11.23 (34). All primers were obtained from Research Genetics, with the exception of *ELN Mva*I, which was synthesized elsewhere.

Between 200 and 500 ng of DNA were amplified with the indicated primers for chromosome 7 in a volume of 25 µl. PCR amplification was performed on a Perkin-Elmer 9600 with 32 cycles of 30 s at 94°C, 45 s at 55–60°C for the annealing, and 80 s at 72°C for the extension. The reaction product was mixed in an equal volume of urea loading buffer (42% urea, 0.1% xylene cyanol, 0.1% bromphenol blue, and 0.1% 0.5 M EDTA) and was loaded onto a 0.4 mm thick 6% polyacrylamide/50% urea gel. Visualization of bands was done by silver staining of the gels.

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